PB83-194522

Adaptation of Natural Microbial Communities to Degradation of Xenobiotic Compounds: Effects of Concentration, Exposure Time, Inoculum, and Chemical Structure

Gulf Coast Research Lab.
Ocean Springs, MS

Prepared for

Environmental Research Lab.
Gulf Breeze, FL

3 Oct 82

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-83-010	2. Journal Article
3. RE PB83-194522	
4. TITLE AND SUBTITLE ADAPTATION OF NATURAL MICROBIAL COMMUNITIES TO DEGRADATION OF XENOBIOTIC COMPOUNDS: EFFECTS OF CONCENTRATION, EXPOSURE TIME, INOCULUM, AND CHEMICAL STRUCTURE	5. REPORT DATE 1983
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) J.C. Spain and P.A. Van Veld	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Gulf Coast Research Laboratory Ocean Springs, MI 39564	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO. 68-D1-5043
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory Office of Research and Development Gulf Breeze, FL 32561	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA/600/04

15. SUPPLEMENTARY NOTES

Applied and Environmental Microbiology, 45(2):428-435, February 1983

16. ABSTRACT

ABSTRACT

Adaptation of microbial populations to degrade xenobiotic compounds faster after exposure to the compound was studied in eco-cores. Radiolabeled test compounds were added to cores that contained natural water and sediment samples. Adaptation was detected by comparing mineralization rates or disappearance of parent compound in pre-exposed and unexposed cores. Microbial populations in pre-exposed cores from a number of freshwater sampling sites adapted to degrade p-nitrophenol faster; populations from estuarine or marine sites did not show any increase in rates of degradation as a result of pre-exposure. Adaptation was maximal after two weeks and not detectable after six weeks. A threshold concentration of 10 ppb was noted, below which no adaptation was detected. With concentrations of 20 to 100 ppb, the biodegradation rates in pre-exposed cores were much higher than in control cores and were proportional to respike concentration. In addition, trifluralin, 2,4-dichlorphenoxyacetic acid (2,4-D) and p-cresol were tested to determine whether pre-exposure affected subsequent biodegradation. Microbial populations did not adapt to trifluralin. Adaptation to 2,4-D was similar to that with nitrophenol. p-Cresol was mineralized rapidly in both pre-exposed and unexposed populations.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 9
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA-600/J-83-010
JOURNAL ARTICLE

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1983, p. 428–435
0099-2240/83/020428-08$2.00/0

Vol. 45, No. 2

Adaptation of Natural Microbial Communities to Degradation of Xenobiotic Compounds: Effects of Concentration, Exposure Time, Inoculum, and Chemical Structure†

JIM C. SPAIN* AND P. A. VAN VELD

U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida 32561 and Gulf Coast Research Laboratory, Ocean Springs, Mississippi 39564

Received 24 May 1982/Accepted 3 October 1982

Adaptation of microbial communities to faster degradation of xenobiotic compounds after exposure to the compound was studied in ecocores. Radiolabeled test compounds were added to cores that contained natural water and sediment. Adaptation was detected by comparing mineralization rates or disappearance of a parent compound in preexposed and unexposed cores. Microbial communities in preexposed cores from a number of freshwater sampling sites adapted to degrade *p*-nitrophenol faster; communities from estuarine or marine sites did not show any increase in rates of degradation as a result of preexposure. Adaptation was maximal after 2 weeks and was not detectable after 6 weeks. A threshold concentration of 10 ppb (10 ng/ml) was observed; below this concentration no adaptation was detected. With concentrations of 20 to 100 ppb (20 to 100 ng/ml), the biodegradation rates in preexposed cores were much higher than the rates in control cores and were proportional to the concentration of the test compound. In addition, trifluralin, 2,4-dichlorophenoxyacetic acid, and *p*-cresol were tested to determine whether preexposure affected subsequent biodegradation. Microbial communities did not adapt to trifluralin. Adaptation to 2,4-dichlorophenoxyacetic acid was similar to adaptation to nitrophenol. *p*-Cresol was mineralized rapidly in both preexposed and unexposed communities.

Laboratory studies of pollutant biodegradation are best conducted by using mixed culture systems taken from the field. The goal of such studies is to use degradation rates measured in the laboratory to predict degradation rates in the environment. A number of factors, such as temperature, salinity, pH, redox potential, microbial biomass, and prior exposure, can affect the degradation rate and, thus, the fate of a toxicant. If laboratory studies are to be used to predict biodegradation rates in the field, it is important that the rate-determining factors be understood.

Previous work has shown that adaptation of microorganisms can play a major role in determining biodegradation rates (3, 4, 6, 7, 9). Adaptation is defined as a change in the microbial community that increases the rate of transformation of a test compound as a result of a prior exposure to the test compound. This is an operational definition, and no attempt is made to distinguish among mechanisms, such as gene transfer or mutation, enzyme induction, and population changes (7). It is likely that each of these mechanisms contributes to the changes in biodegradation rates observed after natural microbial communities are exposed to new substrates. Changes in biodegradation rates due to perturbation of the system during sample collection or subsequent laboratory manipulation are excluded from consideration by the use of proper controls.

The effects of adaptation can be dramatic (7). Even when xenobiotic compounds are added at concentrations below 100 ppb (100 ng/ml), the degradation rates can be 1,000-fold higher in populations that are preexposed to the compound. The study of Spain et al. (7) raised several questions concerning the duration, concentration dependence, site dependence, and compound dependence of microbial adaptation. In this report we describe attempts to answer these questions.

MATERIALS AND METHODS

Test system. Biodegradation of test compounds and adaptation of microbial communities were measured in ecocores as described previously (7). The ecocore test systems were filled with sediment and water from the test sites. Samples from all but the shallowest sites were collected by divers. Radiolabeled test com-

† Contribution 440 from the Gulf Breeze Laboratory.

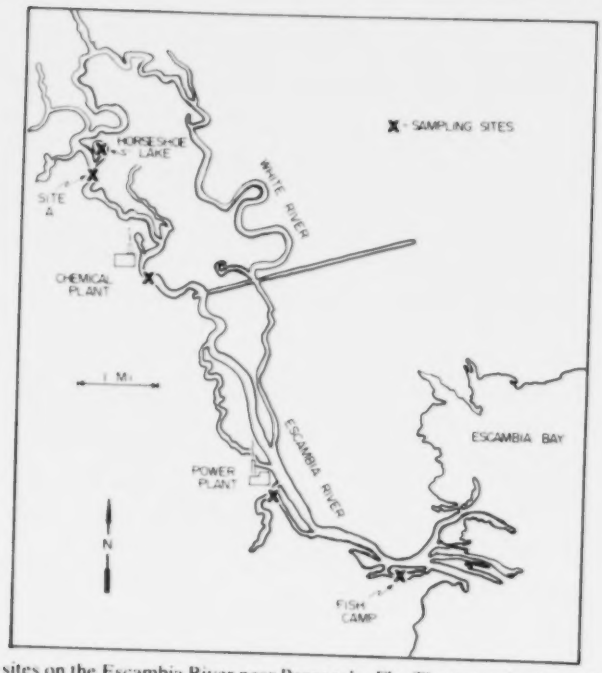

FIG. 1. Sampling sites on the Escambia River near Pensacola, Fla. The water depth at all sites was between 1 and 2 m. Mi, Mile.

pounds were added without carrier, and parent compound disappearance and mineralization were monitored. Adaptation was demonstrated by measuring the degradation of the test compounds in cores preexposed to the compounds for appropriate intervals. The degradation rates in preexposed cores were then compared with the degradation rates in control cores, and differences were taken to indicate adaptation.

Extraction and analysis. Aqueous samples containing *p*-nitrophenol (PNP), 2,4-dichlorophenoxyacetic acid (2,4-D), and *p*-cresol were acidified with concentrated HCl (pH 1 to 2) and extracted with equal volumes of ethyl acetate. Extracts were filtered through Fluoropore filters (pore size, 0.5 μm; Millipore Corp, Bedford, Mass.) and analyzed by high-pressure liquid chromatography. High-pressure liquid chromatographic analyses were performed with a Waters Associates liquid chromatograph equipped with a radial compression column and a UV absorbance detector. Samples that contained radiolabeled test compounds were supplemented with unlabeled compounds at a concentration of 50 mg/liter to allow detection by UV absorbance. Peaks that contained radioactivity were collected, and the radioactivity was measured by liquid scintillation counting. PNP was separated on a silica gel column by using chloroform-methanol-acetic acid (160:40:1) as the mobile phase. UV absorbance was monitored at 254 nm. *p*-Cresol was separated by reverse-phase chromatography, using an octadecylsilane-bonded silica column and methanol-phosphate buffer (80:20) as the mobile phase. The phosphate buffer was 0.1 M phosphoric acid adjusted to pH 3.5 with sodium hydroxide. *p*-Cresol was detected by following absorbance at 280 nm. 2,4-D was separated by reverse-phase chromatography, using acetonitrile–1.0% acetic acid (1:1) as the mobile phase. UV absorbance was measured at 280 nm.

Trifluralin was extracted from aqueous solutions with equal volumes of hexane, and the extracts were analyzed by gas chromatography. Gas chromatographic analyses were performed with a Hewlett Packard model 5830 gas chromatograph equipped with an electron capture detector. The column (2 mm by 2 m) was packed with 3% OV-101 on Chromosorb HP (80/100 mesh) with support-bonded Carbowax-20M. The column temperature was 155°C, and the flow rate of the argon-methane (90:10) carrier gas was 31 cm^3/min.

Culture media and conditions. The culture media and the methods used for enumeration and selection of bacteria have been described previously (7).

Materials. PNP was obtained from Matheson, Coleman and Bell Inc., Norwood, Ohio; *p*-cresol was from Aldrich Chemicals, Milwaukee, Wis.; ααα-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine (trifluralin), 2,4-D, and 2,4-dichlorophenol were from the U.S. Environmental Protection Agency, Research Triangle Park, N.C.; [*U*-^{14}C]PNP and *p*-[ring-*U*-^{14}C]cresol were from Pathfinders, St. Louis, Mo.; [ring-*U*-^{14}C]2,4-D was from Calbiochem, La Jolla, Calif.; and [ring-*U*-^{14}C]trifluralin was from New England Nuclear Corp., Boston, Mass. The purity of chemicals was verified by gas chromatography or high-pressure liquid chromatography before use.

Radioactivity measurements. Radioactivity was mea-

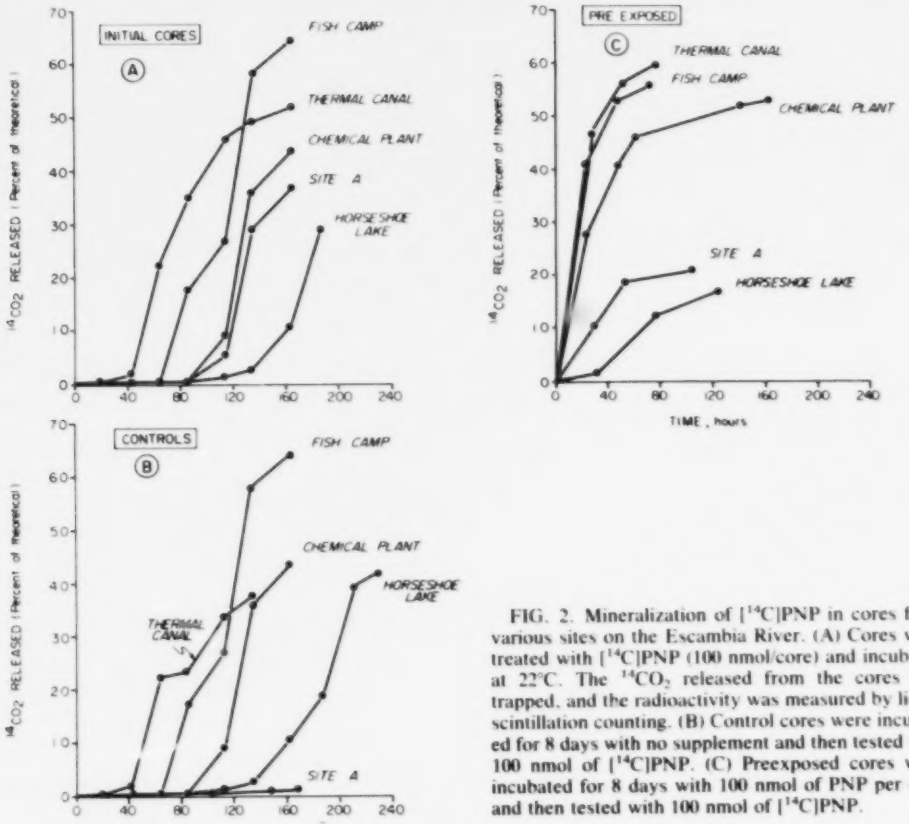

FIG. 2. Mineralization of [^{14}C]PNP in cores from various sites on the Escambia River. (A) Cores were treated with [^{14}C]PNP (100 nmol/core) and incubated at 22°C. The $^{14}CO_2$ released from the cores was trapped, and the radioactivity was measured by liquid scintillation counting. (B) Control cores were incubated for 8 days with no supplement and then tested with 100 nmol of [^{14}C]PNP. (C) Preexposed cores were incubated for 8 days with 100 nmol of PNP per core and then tested with 100 nmol of [^{14}C]PNP.

sured by liquid scintillation counting as described previously (7).

RESULTS

Effect of sampling site. Preliminary work (7) demonstrated that microbial communities from a test site on the Escambia River adapted to degrade PNP faster after an initial exposure to the nitro compound. To determine whether communities taken from other freshwater sites could adapt to mineralize PNP, ecocore samples were collected at five sites on the Escambia River (Fig. 1). The mineralization data for cores treated initially with radiolabeled PNP (initial cores) are shown in Fig. 2A. After a lag period during which mineralization was negligible, the rates of $^{14}CO_2$ release increased substantially in all samples. When PNP was added to control cores after 8 days of incubation, the lag period and subsequent mineralization rates were virtually identical to those of the initial cores (Fig. 2B). In contrast, when cores preexposed to unlabeled PNP were exposed to [^{14}C]PNP, communities from all sites were clearly adapted to degrade the nitro compound (Fig. 2C). Most of the communities adapted to a greater extent than the communities at the original test site (Fig. 1, site A).

To test whether adaptation occurred in estuarine and marine communities, seven sites in the Escambia River estuary and surrounding area (Fig. 3) were sampled and tested for adaptation to PNP. The original river test site (Fig. 1, site A) provided a freshwater control sample; the other test sites sampled were in Escambia Bay, the Gulf of Mexico, and Range Point saltmarsh in Santa Rosa Sound. Only the initial cores were used (Fig. 4) because there was no evidence of mineralization in any of the saltwater samples. The river sample was the only sample that caused any appreciable mineralization of PNP. With the Escambia River core, there was a dramatic increase in the rate of $^{14}CO_2$ release after 4 days of incubation. The bacteria in ecocores were enumerated at the beginning of the experiment (Table 1). There was no apparent

FIG. 3. Test sites in the Escambia River estuary and adjacent areas. Site 1 is on the Escambia River; sites 2, 3, and 5 are in Pensacola Bay; site 6 is in Range Point saltmarsh; and sites 4 and 7 are in the Gulf of Mexico. Mi, Mile.

correlation between mineralization rate and total bacterial biomass. Attempts to isolate PNP-degrading bacteria from the estuarine and saline sites failed. In contrast, PNP degraders were readily isolated from the river test site (7). Subsequent experiments in which preexposed and control cores from the same sites were tested with [^{14}C]PNP gave identical results, and there was no evidence of adaptation in samples from saline environments.

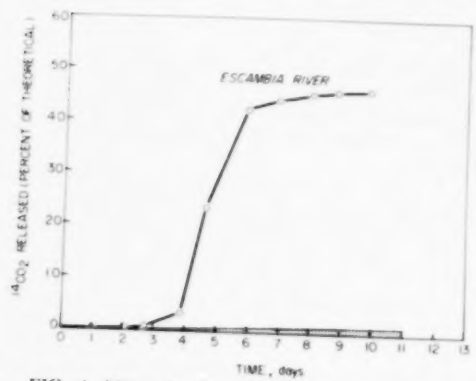

FIG. 4. Mineralization of PNP in ecocores from diverse sampling sites on the Escambia River estuary and nearby Gulf of Mexico (see Fig. 3). Cores were treated with 1.1 μM [^{14}C]PNP and incubated at 20°C. $^{14}CO_2$ was trapped, and the radioactivity was measured by liquid scintillation counting. Data are averages from duplicate cores. The upper curve represents data from Escambia River cores. The mineralization rates in cores from all other sites (lower curve) were indistinguishable.

Duration of adaptation. To determine whether adaptation is important in the environment, it is necessary to know how long communities remain adapted after exposure to a xenobiotic compound. We designed an experiment to determine how long adaption persists after exposure of river communities to PNP. A group of ecocore samples from the Escambia River were treated with unlabeled PNP. A similar group of control cores received no treatment. After incubation for different intervals, [^{14}C]PNP was added to preexposed and control cores, and mineralization was studied by measuring $^{14}CO_2$ release (Fig. 5). The initial rate of $^{14}CO_2$ release was used as a measure of adaptation. No miner-

TABLE 1. Bacterial biomass in ecocores from various sites on the Escambia River estuary and the nearby Gulf of Mexico

Site no.[a]	Site	Water depth (m)	Total bacteria (CFU/ml, $\times 10^6$)
1	Escambia River	2.0	4.9
2	Pensacola Bay, Bayou Chico	6.0	18.4
3	Pensacola Bay	12.0	4.5
4	Gulf, Nearshore	6.0	0.42
5	Pensacola Bay Bridge	4.6	4.4
6	Range Point saltmarsh	0.6	9.8
7	Gulf, Offshore	24.0	0.81

[a] See Fig. 3 for precise locations.

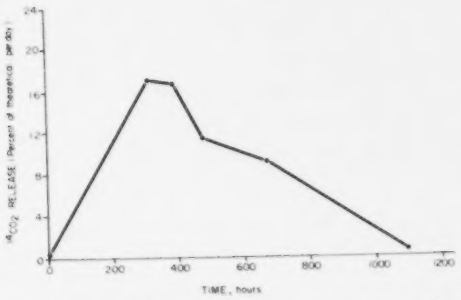

FIG. 5. Duration of adaptation. Cores were taken from the Escambia River sampling site, preexposed to 1.08 μM PNP, and tested with [^{14}C]PNP after 0, 313, 383, 473, 669, and 1,097 h. The $^{14}CO_2$ released from the cores in the first 36 h of the test was trapped and measured by scintillation counting.

alization was detected in the control cores during the course of the experiment. Adaptation was maximal 2 weeks after exposure and fell slowly until it was no longer detectable after 7 weeks. Because bacterial activity in the cores incubated for the longer times was likely to have been less than optimal, adaptation in the environment would probably be different. Therefore, the times indicated by a static experiment such as the one described here would underestimate the duration of adaptation in natural systems.

Concentration effects. To determine whether the degree of adaptation depended on the concentration of PNP used during preexposure or on the concentration used in the biodegradation assay, ecocores were prepared with sediment and water from the Escambia River test site and treated in the following ways: (i) different preexposure concentrations and test concentrations the same as the preexposure concentrations (cores were preexposed to different concentrations of PNP, incubated for 5 days to allow communities to adapt, and then tested with radiolabeled PNP at the same concentrations used for preexposure); (ii) different preexposure concentrations and a test concentration of 0.72 μM (cores were preexposed to different concentrations of PNP and incubated for 5 days, and then 0.72 μM radiolabeled PNP was added to each core); and (iii) 0.72 μM PNP preexposure and different test concentrations (cores were preexposed to 0.72 μM PNP, incubated for 5 days, and then tested with different concentrations of radiolabeled PNP).

The rate of mineralization of PNP in adapted cores (Fig. 6A) depended on the PNP concentrations used for preexposure and biodegradation tests. The mineralization rates in ecocores preexposed to identical concentrations and tested at different concentrations depended on the concentration of PNP used in the biodegradation test (Fig. 6B).

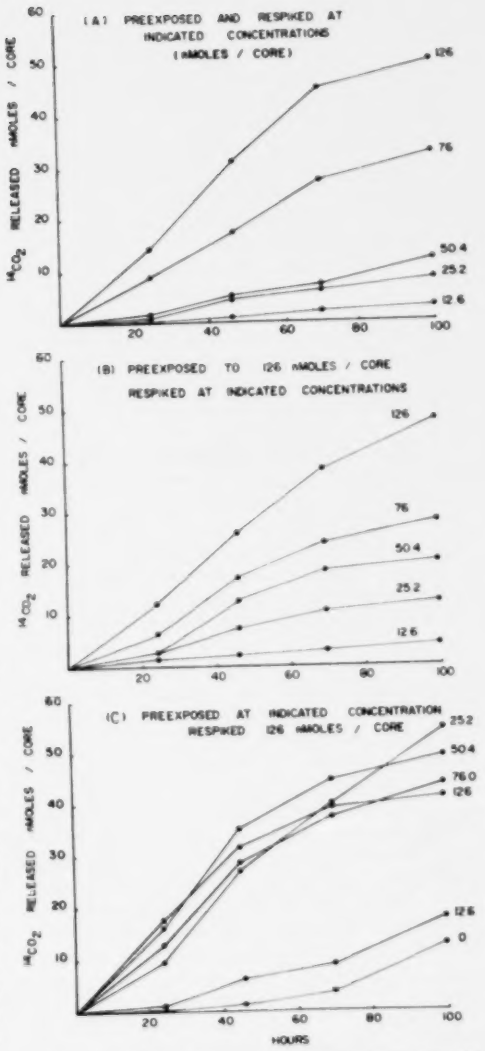

FIG. 6. Effects of concentration on adaptation. (A) Cores were preexposed to unlabeled PNP at the indicated concentrations (nanomoles per core) for 6 days and then tested with [^{14}C]PNP at the same concentrations, and the $^{14}CO_2$ released from the cores was trapped and measured. (B) Cores were preexposed to 126 nmol of PNP per core for 6 days and then tested with [^{14}C]PNP at the indicated concentrations. (C) Cores were preexposed to PNP at the indicated concentrations for 6 days and then tested with 126 nmol of [^{14}]PNP.

Communities preexposed to different concentrations and tested at the same concentrations

mineralized PNP at similar rates when preexposure concentrations were 25.2 nmol/core or greater (Fig. 6C). Preexposure concentrations influenced the rate to some extent; there was a slight lag in mineralization rate in communities preexposed to 25.2, 50.4, or 76 nmol/core, but after 70 h the total amounts of $^{14}CO_2$ released were similar. Communities in control cores that received no preexposure gave no evidence of adaptation to faster degradation of the nitro compound, and communities preexposed to 12.6 nmol/core showed only slight adaptation when they were tested with 126 nmol/core.

Each core in the experiment described above was matched with two identically treated cores in which both parent compound disappearance and $^{14}CO_2$ release were followed. The results confirmed the mineralization data shown in Fig. 6.

Adaptation with other compounds. Trifluralin, PNP, *p*-cresol, and 2,4-D were tested in the ecocore system to determine whether the microbial communities from the river test site could adapt to degrade other compounds. For each compound, two cores were supplemented initially with ^{14}C-labeled compound; one of these was used to follow mineralization, and the other was sampled at intervals to measure the concentration of the parent compound in the water. Three cores were treated with unlabeled test compound, and three control cores received no addition. Mineralization in the cores treated initially with radiolabeled *p*-cresol was rapid and immediate. With 2,4-D and PNP, there was a lag period before rapid mineralization occurred. No significant mineralization was detected in the cores that contained trifluralin. After the rates of mineralization began to decrease in the initial cores that contained PNP, *p*-cresol, or 2,4-D, ^{14}C-labeled test compounds were added to control and preexposed cores. Samples were removed from two control and two preexposed cores for analysis of the parent compound. The $^{14}CO_2$ released from all three cores was trapped and used as a measure of mineralization.

Cores preexposed to PNP rapidly released $^{14}CO_2$, and there was a concomitant, rapid disappearance of the parent compound (Fig. 7A). Mineralization and disappearance of the parent compound were much slower in control cores. Similar results were obtained with 2,4-D (Fig. 7B), and there was no accumulation of 2,4-dichlorophenol.

Cores tested with *p*-cresol gave identical rates of mineralization and parent compound disappearance regardless of whether they were preexposed (Fig. 7C). In both cases, *p*-cresol was attacked rapidly by the microbial community.

Trifluralin was degraded only very slowly, and there were no differences between rates in preexposed communities and those in control communities (Fig. 7D). No radioactivity was released as $^{14}CO_2$.

DISCUSSION

Our results indicate that adaptation of aquatic microbial communities can last for several weeks after exposure to a xenobiotic compound. Therefore, adaptation must be considered when we predict the fate of xenobiotic compounds in environments subjected to repeated or extended exposures. Biodegradation tests should be done with adapted microbial communities, and compounds that cause adaptation can be expected not to persist. There is considerable evidence to indicate that such is the case in terrestrial systems as well (6).

The ability of communities to adapt varies widely from site to site. Fresh- and saltwater communities clearly differ in the ability to mineralize PNP. It is possible that, given sufficient time, organisms from saline environments would also adapt to degrade nitrophenol faster after exposure, but we have been unable to demonstrate such a response.

Baughman et al. (1) have shown that for many compounds biodegradation rates are proportional to the total bacterial biomass. This relationship does not seem to be true for compounds that are attacked by only a few specific organisms in the test system. Our results strongly suggest that second-order rate constants based on total bacterial biomass cannot be used to predict the biodegradation of certain pollutants that cause adaptation of the microbial community. Preliminary evidence suggests that the response of a community is governed by the presence or absence of bacteria that are able to use the nitro compound as a carbon source.

Results obtained with different concentrations of PNP indicate that the extent of adaptation achieved by a microbial community is not directly proportional to the preexposure concentration of the substrate. There seems to be a threshold concentration of PNP below which there is no detectable adaptation of the community. The threshold concentration is similar to the concentration indicated in our previous work (7). Active microbial communities were produced by preexposure to PNP concentrations above the threshold, and higher preexposure concentrations caused only slight increases in biodegradation rates. The mineralization rates shown by adapted communities were influenced more by the test concentration than by the preexposure concentration within the range tested. Future studies on the practical applications of this observation should enable us to predict the fate of organic pollutants more accurately. Prediction of microbial adaptation after exposure to a toxi-

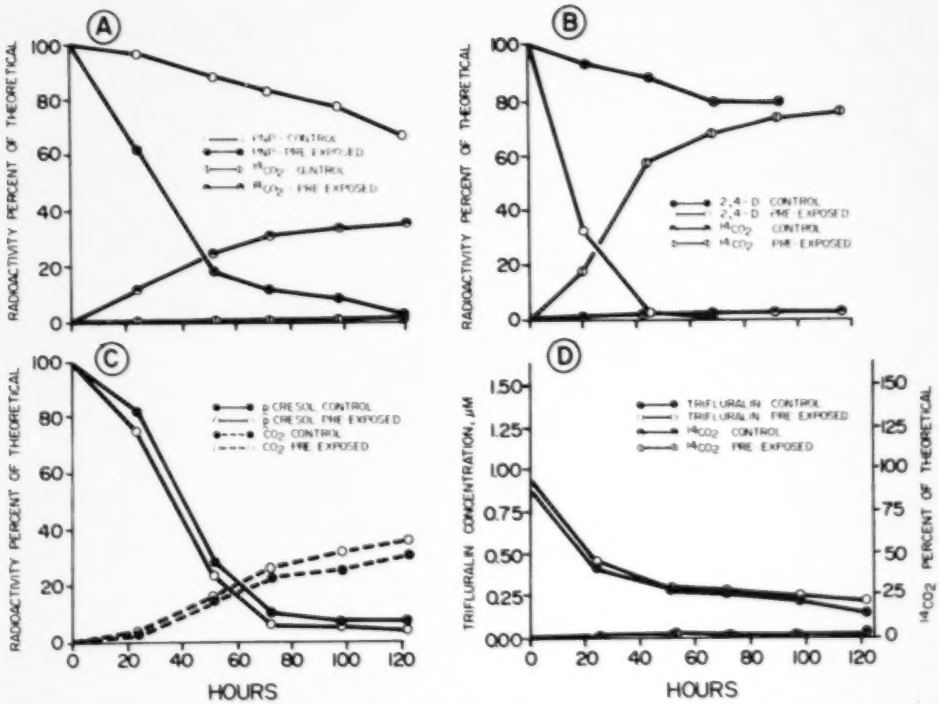

FIG. 7. Mineralization of radiolabeled test compounds by preexposed and control populations from the Escambia River. (A) PNP. (B) 2,4-D. (C) *p*-Cresol. (D) Trifluralin. Cores from the Escambia River test site were exposed to the test compounds at concentrations of 1.08 μM; control cores received no supplement. After 8 days of incubation at 20°C, all cores were tested with 1.08 μM radiolabeled compound. During subsequent incubation $^{14}CO_2$ release was measured, and water samples were analyzed for parent compounds by high-pressure liquid chromatography or gas chromatography. The values shown are averages of data from duplicate cores; sample-to-sample variation never exceeded 10% of the mean.

cant will become easier if the extent of adaptation is the same over a relatively wide range of exposure concentrations. Furthermore, priming of microbial communities near potential pollutant discharge sites with small amounts of the pollutant might be used to ensure a rapid microbial response after a spill.

The results of the experiments with various test compounds provide examples of several ways that microbial communities can respond in an adaptation test. The microbiota clearly degraded PNP faster as a result of preexposure. There was a lag period of several days before communities exposed to PNP began to attack the test compound, but communities in cores tested after the adaptation period attacked PNP immediately. The data from the 2,4-D experiments lead to similar conclusions.

When *p*-cresol was added to ecocores, the results were markedly different. There was no lag period before the population attacked *p*-cresol, and preexposure in the laboratory did not alter biodegradation rates. The following explanations for such a rapid attack are possible: (i) the adaptation may have been extremely fast and thus may not have been detected in our experiment; (ii) the enzymes for *p*-cresol degradation may have been constitutive in the community; or (iii) the community in the river may have been exposed to *p*-cresol or a similar compound before the samples were collected, which would have produced microbial populations able to degrade *p*-cresol immediately. Such exposure could be chronic at the test site, due to the presence of cresols or similar compounds in runoff from agricultural land and forests.

Trifluralin degradation was relatively slow, and there was no detectable adaptation. Therefore, the disappearance of this compound was due to abiotic factors or to biodegradation by nonspecific cooxidative processes (5).

The results described above would be useful in predicting the fate of the four test compounds if the compounds were released in the river.

Trifluralin would biodegrade slowly and at a relatively constant rate, depending on the concentration. *p*-Cresol would degrade rapidly and immediately, whereas PNP and 2,4-D would persist during adaptation of the microorganisms and then biodegrade rapidly.

Measurements of mineralization do not always accurately reflect the disappearance of a parent compound in a biodegradation test (1). The results of our experiments clearly show that for PNP and *p*-cresol, mineralization rates closely parallel rates of parent compound disappearance. This is probably true in most cases where the initial step is rate limiting in a biodegradation sequence that involves complete mineralization. Degradation of the above compounds begins with incorporation of molecular oxygen into the substrate (2, 8); subsequent steps in the pathway are considerably less complex. In the case of trifluralin, the initial transformation probably does not produce readily degradable products; therefore, mineralization is a poor indicator of biotransformation.

ACKNOWLEDGMENTS

We thank the dive team of the U.S. Environmental Protection Agency Environmental Research Laboratory, Gulf Breeze, Fla., for collecting ecocore samples used in this study.

This work was funded in part by U.S. Environmental Protection Agency contract 68-D1-5043 with Battelle Columbus Laboratories and subcontract T-6423 (7197)-037 with Gulf Coast Research Laboratory, Ocean Springs, Miss.

LITERATURE CITED

1. **Baughman, G. L., D. F. Paris, and W. C. Steen.** 1980. Quantitative expression of biotransformation rate, p. 105–111. *In* A. W. Maki, K. L. Dickinson, and J. Cairns, Jr. (ed.), Biotransformation and fate of chemicals in the aquatic environment. American Society for Microbiology, Washington, D.C.
2. **Dagley, S., and M. D. Patel.** 1957. Oxidation of *p*-cresol and related compounds by a *Pseudomonas*. Biochem. J. **66**:227–233.
3. **Felset, A., J. V. Maddox, and W. Bruce.** 1981. Enhanced microbial degradation of carbofuran in soils with histories of Furadan use. Bull. Environ. Contam. Toxicol. **26**:781–788.
4. **Fournier, J. C., P. Codaccioni, and G. Soulas.** 1981. Soil adaptation to 2,4-D degradation in relation to the application rates and the metabolic behavior of the degrading microflora. Chemosphere **10**:977–984.
5. **Perry, J. J.** 1979. Microbial cooxidations involving hydrocarbons. Microbiol. Rev. **43**:59–72.
6. **Simon-Sylvestre, G., and J. C. Fournier.** 1979. Effects of pesticides on the soil microflora. Adv. Agron. **31**:1–81.
7. **Spain, J. C., P. H. Pritchard, and A. W. Bourquin.** 1980. Effects of adaptation on biodegradation rates in sediment/water cores from estuarine and freshwater environments. Appl. Environ. Microbiol. **40**:726–734.
8. **Spain, J. C., O. Wyss, and D. T. Gibson.** 1979. Enzymatic oxidation of *p*-nitrophenol. Biochem. Biophys. Res. Commun. **88**:634–641.
9. **Torstensson, N. T. L., J. Stark, and B. Goransson.** 1975. The effect of repeated applications of 2,4-D and MCPA on their breakdown in soil. Weed Res. **15**:159–164.